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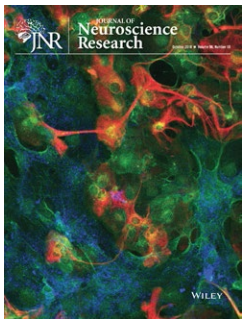
REVIEW



Nucleus–cytoplasm cross-talk in the aging brain

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Abstract

Aging is a primary risk factor for fatal neurodegenerative disorders, yet the mechanisms underlying physiological healthy aging and pathological aging, and how these mechanisms can divert one scenario to the other, are not completely understood. In recent years, reports indicate that alterations in nucleocytoplasmic transport may be a hallmark of both healthy and pathological aging. In this review, I summarize recent evidence supporting this information, specifically focusing on the association between the nucleocytoplasmic transport and aging of the brain, indicating both common and case-specific mechanisms and their interplay, and pointing out alterations of these mechanisms as regulatory "switches" for the fate of the aging brain. Importantly, some of these alterations are intervenable druggable targets, paving the way to a future pharmacotherapeutic intervention.

KEYWORDS

aging, amyotrophic lateral sclerosis, Alzheimer's disease, frontotemporal dementia, Huntington disease, neurodegeneration, neurodegenerative diseases, prion diseases, tauopathies

1 | INTRODUCTION

Aging is a multifactorial condition associated with complex changes occurring virtually in every cell of our body (Kirkwood, 2008; López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013). Changes underlying aging are characterized by the chronic accumulation of molecular and cellular damage throughout the lifespan, and by the following cellular response to stress by activating mechanisms that support cellular functions and hence maintain cellular homeostasis. Indeed, organisms have developed different mechanisms to counteract the potentially deleterious effects of aging to maintain their homeostasis and increase their lifespan and functionality, but in the end, aging is a normal stage of life, and all organisms must obey to it.

As the brain ages, different chemical, structural, and functional changes occur, that eventually translate into changes in cognitive function (Mattson & Magnus, 2006). Among the alterations that occur in the brain with aging, alteration of brain proteome homeostasis is one of the best described. For example, differences between young and old brain proteomes can subsist at different levels, such as differences in the magnitude of protein expression, post-translational modifications, and protein folding stability (Roberts, Liu, Karnuta, & Fitzgerald, 2016; Stauch, Purnell, Villeneuve, & Fox, 2015; Vanguilder & Freeman, 2011). Additionally, recent evidence shows how alteration of the brain proteome that occurs with aging also include changes in the subcellular localization of proteins between the young and old brain. Age-dependent differences in subcellular localization of proteins, specifically changes in cytosolic-nuclear localization, have been linked to decreased neuronal functionality and, consequently, to an increased propensity of developing neurological disorders. Therefore, in this mini-review, I will focus on the changes in cytosolic-nuclear localization of proteins, and in the alteration of the nucleocytoplasmic transport, which occur during brain aging, and its potential effect on neurodegenerative disorders.

2 | AGE-DEPENDENT ALTERATION IN NUCLEAR IMPORT–EXPORT FUNCTIONALITY

The trafficking of proteins and large molecules between the cytoplasm and the nucleus is mainly governed by the nuclear pore complexes (NPCs), large protein complexes that span the nuclear

Significance

Alteration of nucleocytoplasmic transport is increasingly reported as a major mechanism of loss of homeostasis and neurotoxicity during aging and neurodegeneration. Age-dependent increase in oxidative stress, perturbation of proteostasis, accumulation of protein aggregates, together with the uniqueness of neurons as nondividing, long-living cells in the body, are among the main causes for the improper nucleocytoplasmic transport observed during aging. This review tries to combine the main evidences for all the above-mentioned points, in order to clarify potential interrelated mechanisms and to provide a wider scenario of the nucleocytoplasmic transport defects in an aging neuron.

envelope (Wente & Rout, 2010). The functions of the NPCs and of the nuclear envelope go far beyond the simple regulation of trafficking macromolecules, and have been implicated in other unrelated cellular processes, including cell cycle progression, regulation of gene expression, and anchoring sites for chromosomes and cytoskeletal components to the nuclear periphery (reviewed by (D'Angelo & Hetzer, 2006)). NPCs in postmitotic neurons do not turn over and are extraordinarily long-lived (D'Angelo, Raices, Panowski, & Hetzer, 2009; Savas, Toyama, Xu, Yates, & Hetzer, 2012). Without mitotic turnover, NPC components become oxidized after exposure to aging-derived toxic metabolites; this results in the progressive deterioration of NPC function, and the consequent leakage of cytosolic proteins into the nuclear compartment of neurons (D'Angelo et al., 2009). A study to analyze differences in proteome between young and old brains in rats reports an altered cytoplasmic-nuclear localization of proteins (Ori et al., 2015). In this study, the authors fractionated the homogenates in different subcellular fractions (among which nuclei, postnuclear membranes, and cytoplasmic fractions) prior to spectrometric analysis of young and old rat brains lysates. The authors identified that seven proteins in the brain had opposite subcellular distribution (i.e., an increased abundance in one compartment counterbalanced by a decrease in another) between young and old brains. As this change could not be explained by changes in translation output, the authors therefore interpreted such data as indicative of a real change in subcellular localization that occurs with aging of the brain. The differentially redistributed proteins include protein and RNA modifying enzymes, proteins involved in translation, and nuclear transport factors. For example, the authors identified that Exportin-5, a NPCs-interacting protein involved in the export of miRNAs (Lund, Güttinger, Calado, Dahlberg, & Kutay, 2004) and tRNAs (Calado, Treichel, Müller, Otto, & Kutay, 2002), shows an increased abundance in the cytoplasm of cells from old brains, implying a potential alteration of nuclear transport activity. These data, in accordance with the previously described age-dependent deterioration of the NPC (D'Angelo et al., 2009), indicate a modification of protein and RNA transport machineries associated with brain aging.

3 | NPCS, NUCLEAR LAMINA, AND OXIDATIVE STRESS

The correct functioning of NPC is also strictly dependent on the integrity of the nuclear lamina, a fibrillar protein structure lining the inner surface of the nuclear envelope important to maintain nuclear stability, organize chromatin, and bind NPCs (Cobb et al., 2016; D'Angelo et al., 2009; Sakuma & D'Angelo, 2017). Alteration of the nuclear lamina is strongly associated with premature aging and diseases.

3.1 | Hutchinson-Gilford progeria syndrome and nucleocytoplasmic transport alteration

Mutations of the gene *LMNA* coding for Lamin A are associated with premature aging syndromes, such as Hutchinson-Gilford progeria syndrome (HGPS) (Liu, Barkho, et al., 2011a; Liu, Suzuki, et al., 2011b). HGPS is an extremely rare genetic disorder characterized by the rapid appearance of aging beginning in childhood. Affected children develop a characteristic facial appearance (small chin, prominent eyes, protruding ears). Additional typical features include cardiovascular disease and stroke, atherosclerosis, hip dislocations, and other abnormalities. Individuals with HGPS die of heart disease at an average age of 13 years, with a range of about 8 to 21 years (Piekarowicz, Machowska, Dzianisava, & Rzepecki, 2019; Ullrich & Gordon, 2015). Mutant lamins trigger nuclear lamina abnormalities, make the nuclear envelope unstable, and progressively damages the nucleus, in turn making cells more likely to die prematurely (Prokocimer, Barkan, & Gruenbaum, 2013). Accumulation of a truncated form of Lamin A results also in dysfunctional nuclear import (Kelley et al., 2011) and an increase in oxidative stress (Richards, Muter, Ritchie, Lattanzi, & Hutchison, 2011). An increase in oxidative stress is a well-described age-related factor, and is one of the most important key events in inducing both (physiological) organism aging and (pathological) neurodegeneration (Bokov, Chaudhuri, & Richardson, 2004; Cui, Kong, & Zhang, 2012; Mattson & Magnus, 2006; Stranahan & Mattson, 2012). In young cells, a physiological composition of the nuclear envelope is fundamental to counteract oxidative stress. For example, conserved cysteine residues in the Lamin A tail domain are targets for irreversible oxidative damage during replicative senescence. These cysteine residues work as a "buffer" system of the cell to counteract oxidative stress. Indeed, in the absence of Lamin A or after the loss of the conserved cysteine residues, reactive oxygen species (ROS) basal levels are elevated and cells enter a premature state of senescence following only mild levels of ROS stimulation (Pekovic et al., 2011; Sieprath et al., 2015).

Another example of the interplay between nuclear lamina and oxidative stress is the case of Oct-1. Oct-1 is a transcription factor essential for the regulation of stress response genes to preserve cells from oxidative stress-mediated damage. Oct-1 is also known to interact with Lamins (Malhas, Lee, & Vaux, 2009). Different Oct-1-dependent genes, including a subset of genes involved in

the response to oxidative stress, are dysregulated in cells lacking *LMNB1*. Additionally, Oct-1 binds to the putative octamer-binding sequences of the dysregulated genes, and this activity is increased in cells lacking functional Lamin B1. This indicates that Oct-1 exerts a negative, repressive effect on transcription of oxidative stress response genes. In the absence of Lamin B1 (Malhas et al., 2009) or with senescence-induced decreased levels of Lamin B1 (Shimi et al., 2011), cells have elevated levels of ROS and are more susceptible to oxidative stress. Therefore, Lamin B1 sequesters Oct-1 at the nuclear periphery and prevents it from fully entering the nucleus and exercising its negative transcriptional function. An abnormal efflux of Oct-1 from the nucleus was also observed in cells lacking Lamin A/C (De Vos et al., 2011). In summa, these effects indicate that the nuclear envelope, by physically sequestering transcription factors out of DNA-binding regions, can regulate gene expression and contribute to the cellular response to oxidative stress and aging, and that changes in lamina composition can therefore lead to alterations in nucleocytoplasmic compartmentalization and alter the proper cellular response to stress.

Changes in nucleocytoplasmic compartmentalization may not be solely due to reduced NPC functionality, but also to other factors such as changes in the Ran gradient. The small GTPase Ras-related nuclear protein (Ran) regulates the ability of both importins and exportins to transport their cargo across the nuclear membrane. This transport regulation by the Ran GTPase requires its nuclear localization and GTP loading by the chromatin-associated exchange factor RCC1. In fact, RCC1 is preferentially located inside the nucleus, while Ran GTPase-activating proteins (GAPs) that catalyze the hydrolysis of GTP to GDP are located exclusively in the cytoplasm (Stewart, 2007). This creates a Ran protein gradient used by importins and exportins for a physiologically directional nucleocytoplasmic transport. However, cellular stress such as oxidative stress can disrupt Ran gradient (Stochaj, Rassadi, & Chiu, 2000; Yasuda, Miyamoto, Saiwaki, & Yoneda, 2006). For example, cells from patients with HGPS have elevated ROS and a disrupted Ran protein gradient (Bischoff & Ponstingl, 1991; Datta, Snow, & Paschal, 2014; Kelley et al., 2011; Viteri, Chung, & Stadtman, 2010). Similarly to what occurs with Lamin A (Pekovic et al., 2011; Sieprath et al., 2015), cysteine residues on RCC1 are oxidized in cells exposed to oxidant agents (Chatterjee & Paschal, 2015). The cysteine oxidation reduces RCC1 binding to RanGDP, inhibited nuclear import and disrupt Ran protein gradient. Interestingly, the pharmacological inhibition or depletion of NAT10, a nucleolar acetyltransferase that regulates acetylation of microtubules during cell division (Shen et al., 2009), rescues many phenotypes of cells from patients with HGPS including assembly of the nuclear pore complex, chromatin organization, nuclear Ran localization and import of nuclear proteins (Larrieu, Britton, Demir, Rodriguez, & Jackson, 2014; Larrieu et al., 2018). These results underscore a potential pharmacological therapeutic strategy for HGPS, and consequently also for other age-associated pathologies with impaired nucleocytoplasmic transport.

3.2 | Adult-onset autosomal dominant leukodystrophy: The role of Lamin B in neurodegeneration

The nuclear lamina-dependent effects on transcription are proposed to be at the bases not only of aging-related processes, but also of neurodegenerative states, such as in adult-onset autosomal dominant leukodystrophy (ADLD). ADLD is a very rare demyelinating neuropathy of the central nervous system that presents itself in the fourth or fifth decade of life (Lin, Ptácek, & Fu, 2011; Padiath et al., 2006). This disorder is often associated with early autonomic symptoms preceding ataxia and pyramidal abnormalities. In ADLD patients, defects in brain white matter are observed, particularly in the cerebellum, corticospinal tracts, and corpus callosum, leading to brain and spinal cord atrophy (Sundblom, Melberg, Kalimo, Smits, & Raininko, 2009). ADLD has been shown to be caused by duplications involving the gene *LMNB1* on the chromosome 5q32 (Padiath et al., 2006). The duplication of *LMNB1* gene leads to an increased expression of Lamin B1 protein and mRNA in brain tissue. Increased expression of Lamin B1 results in perturbations of inner nuclear membrane proteins, chromatin organization, and nuclear pore transport (Lin & Fu, 2009). One proposed mechanistic model for the disease is that the increased expression of Lamin B1 suppresses the transcription and the consequent lack of myelin basic protein and proteolipid protein in oligodendrocytes, therefore, leading to central myelin failure (Lin et al., 2011).

In summary, in the context of neuronal aging, the increase in oxidative stress and the consequent build up of damage to nuclear pores and nuclear lamina components that occurs during a lifespan affects the proper nucleocytoplasmic transport by affecting the long-lived nuclear pore proteins as described (D'Angelo et al., 2009). These effects alter the proper cellular transcriptional response that counteracts the increased oxidative stress conditions. As a result, the sum of all these conditions can culminate in a substantial, functional abrogation of NPC function in safeguarding the intranuclear material, paving the way to the pathogenesis of aging-related neuronal dysfunction and, in turn, neurodegeneration.

4 | PROTEIN AGGREGATES AFFECT NUCLEOCYTOPLASMIC TRANSPORT DURING BRAIN AGING AND DISEASE

During aging, proteins are damaged or chemically modified by reactive substances such as oxidants and glycans. Such modified proteins are not usually functional, improperly folded, and potentially toxic to the cell (Höhn, Jung, & Grune, 2014), and are consequently removed by cellular proteolytic systems. However, during aging, the activity of these systems decrease (Bingol & Sheng, 2011; Keller, Gee, & Ding, 2002; Keller, Hanni, & Markesbery, 2000). Together with this, the accumulation of reactive species leads to the accumulation of damaged proteins and the build up of protein aggregates (Nowotny, Jung, Grune, & Höhn, 2014). The accumulation of these aggregates

interferes with the normal cellular homeostasis and affect the whole neuronal metabolism, and is one of the key aspects responsible not only for the increased neuronal vulnerability during aging, but it can also lead to the onset of age-related disorders (David, 2012; Mattson & Magnus, 2006). As neurons are postmitotic cells, they cannot dissipate protein aggregates simply by cell division, and the age-related decline of the degradation systems further enhances the vulnerability of neurons. Additionally, the peculiar polarized structures of neurons (dendrites and axon) can additionally contribute to neuronal vulnerability to altered proteostasis associated with protein aggregates. For example, autophagosomes (a key structure of autophagy-dependent intracellular degradation of proteins) are formed at the distal ends of neurons (Maday, Wallace, & Holzbaur, 2012; Yue, Wang, & Komatsu, 2008) and then undergo retrograde transport to the soma along the axons (Wong & Holzbaur, 2014). As axonal transport activity is essential for supporting physiological neuronal function, perturbation of proteostasis, and the consequent accumulation of protein aggregates can cause a blockage in the traffic of proteins and cargoes in the axon, leading to neuroaxonal dystrophy and, eventually, to neurodegeneration.

Moreover, the physiological aggregation of non-disease-related proteins in the context of aging can lead to the aggregation of disease-related protein, and therefore switch a normal, physiological aging into a pathological aging phenotype (David, 2012; Münch & Bertolotti, 2010). For example, age-dependent physiological aggregates could titrate anti-aggregation factors away from disease-aggregating proteins (Salomons et al., 2009) or could directly induce the aggregation of disease-related proteins by a cross-seeding mechanism (Münch & Bertolotti, 2010; Olzscha et al., 2011).

4.1 | Huntington's disease

Protein aggregates are at the basis of all the major neurodegenerative disorders including Prion disorders, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) (Ross & Poirier, 2004). Among the different types of neurodegeneration-linked aggregates, one of the best characterized to pathologically contribute to the alteration of nuclear transport are the aggregates of the protein Huntingtin (HTT), the pathological hallmark of Huntington's disease (HD). HD is one of the most prevalent dominantly inherited neurodegenerative diseases. HD patients suffer from a clinical triad of movement disorder, psychiatric symptoms, and cognitive impairment. The disease is caused by a CAG repeat expansion encoding an elongated polyglutamine stretch near the N terminus of Huntingtin (Bates et al., 2015). The disease mechanisms downstream of mutant HTT (mHTT) expression suggest that the expansion of polyglutamine trait elicits gain-of-function proteinopathy that may affect both nuclear and cytoplasmic cellular function (Bates et al., 2015). mHTT misfolds and forms aggregates both in the nucleus and in the cytoplasm of affected neurons, and these aggregates are the histopathological hallmark of the disease. Genetic manipulations that force the nuclear translocation of mHTT exacerbate the pathogenesis of the disease in mouse models (Gu et al., 2015). Interestingly, a recent study by Grima and colleagues (Grima et al., 2017) provides evidence of a compromised nuclear pore function in HD models. In this study, the authors test the hypothesis that mHTT may selectively impair specific NPC proteins and soluble transporters. Indeed, they found several nucleoporins (NUPs) and NUP-associated proteins such as RanGAP1 (as in Gasset-Rosa study, discussed below), Nup88, and Nup62 as mislocalized and partially sequestered by mHTT in the striatum and in the cortex of THE R6/2 mouse model of HD and in the postmortem human striatum tissue from HD patients. RanGAP1 is a GTPase-activating protein located on the cytoplasmic filaments of the NPC that is required for nuclear export and import. High levels of Ran-GTP inside the nucleus compared to the cytoplasm is fundamental for a physiological active transport through the NPC (Floch, Palancade, & Doye, 2014). The correct maintenance of the nuclear-cytoplasmic Ran gradient mediated by RanGAP1 is critical for cell survival; indeed, its loss has been demonstrated to induce cell death (Hetzer, Gruss, & Mattaj, 2002). By reducing the levels of mHTT, the authors could normalize RanGAP1 levels. Indeed, intrastriatal injection in R6/2 mice models of HD of microRNA to reduce the levels of PIAS1, an E3-SUMO ligase that modulates SUMO-1 and SUMO-2/3 modification of HTT (O'Rourke et al., 2013; Ochaba et al., 2016), reduces formation of high molecular weight species of mHTT aggregates and restores the levels of nuclear RanGAP1. On the contrary, transient expression of mHTT in primary cortical neurons phenocopies the nuclear pore leakiness, the deficiency in active nuclear transport, and the mislocalization of RanGAP1 as identified in HD. Accordingly, increasing RanGAP1 levels in fly models expressing mHTT reduces neurodegeneration and increases the lifespan. Pharmacological intervention with CRM1 inhibitor KPT-350 or KPT-276 reduced cell death and rescued nucleocytoplasmic trafficking defects in primary cortical neurons, pointing to the inhibition of nuclear export as a neuroprotective mechanism through the compensation of impaired nuclear import in HD.

In support of the findings by Grima and colleagues, a study by Gasset-Rosa and colleagues (Gasset-Rosa et al., 2017) confirmed and expanded the knowledge on an altered nuclear transport in HD. By analyzing the striatum and the cortex (two HD vulnerable brain regions) of different mouse models expressing mHTT, and using HD patients-induced pluripotent stem cells (iPSC)-derived neurons and HD postmortem brains, the authors identified several defects in the nuclear membrane and NPC components, such as altered nuclear membrane shape, mislocalization, and aggregation of the protein RanGAP1, NUP62 and Gle1, an export factor for mRNA. The aggregates of mHTT in the nucleus partially sequester these components, suggesting a potential functional depletion mechanism. Indeed, mRNA was shown to progressively and aberrantly accumulate in the nucleus of HD neurons. Additionally, in HD mouse models the nuclear envelope and lamina are improperly folded, causing alterations both in passive and in active permeability through the NPC, and an increase in DNA double-strand breaks. Aberrant nuclear architecture is a typical cellular hallmark of aging (López-Otín et al., 2013; Scaffidi & Misteli, 2006). In order to investigate whether mHTT

induces nuclear envelope alterations in mice models of HD, the authors analyzed the nuclear shape in cortex and striatum by defining two different types of nuclear morphology: the "normal" morphology, typical of young neurons, where nuclear lamina is predominantly round and regular in shape; and the "altered" morphology, with alterations (from moderate to severe) and with an irregular shape of nuclear lamina showing several invaginations or protrusions. In all the HD models under investigation, the authors identified mHTT-dose-dependent and age-dependent nuclear envelope alterations, with up to 95%–100% of neurons under investigation presenting nuclear architecture abnormalities at late stages of the disease. The authors confirmed the relevance of this finding by analyzing the nuclear morphology within the motor cortex of two HD patients and two control individuals, identifying a significant increased number of misshapen nuclei in HD patients compared to control individuals.

The authors describe the entire scenario as an exacerbated condition of physiological neuronal aging, in which nuclear envelope and lamina show a commencement of improper folding, the pore permeability is slightly increased, mRNAs start to accumulate in the nucleus and RanGAP1 begins to aggregate on the nuclear surface.

In summary, the underlying concept is that, in HD, mHTT is accelerating and exacerbating neuronal dysfunction (i.e., NPC loss-of-functionality, defects in nuclear shape, defects in nucleocytoplasmic compartmentalization) observed during healthy, physiological brain aging.

4.2 | AD and tauopathies

Another disease-related, aggregation-prone protein known to disrupt nucleocytoplasmic transport is protein tau. Tau is the main constituent of neurofibrillary tangles observed in AD. While tau aggregation and phosphorylation are established hallmarks of AD, the mechanisms underlying tau-associated neuronal dysfunction and damage are still uncertain. Very recently, phosphorylated tau was shown to alter nucleocytoplasmic transport by directly interacting with Nup98, a nucleoporin of the NPC, in hippocampal neurons from AD patients (Eftekharzadeh et al., 2018). When interacting with Nup98, tau forces the mislocalization of Nup98 outside the NPC, rendering the nuclear pore leaky. Once in the cytosol, Nup98 interacts directly with phospho-tau and facilitates its aggregation. By knocking down the expression of tau, it was possible to restore Nup98 defects and nucleocytoplasmic defects in the hippocampus of rTg4510 mice, a rodent model of AD. It seems therefore that tau-dependent neurotoxicity (in AD and in other tauopathies) could be at least mediated by phospho-tau-induced NPC dysfunction.

Autosomal-dominant mutations in the *MAPT* gene coding for Tau protein cause a form of inherited frontotemporal dementia (FTD). In a very recent report by Paonessa and colleagues (Paonessa et al., 2019), the authors identify that mutant hyperphosphorylated tau mislocalizes from the axon to the cell body and dendrites in cortical neurons. The new mislocalization of tau in the cell body leads to abnormal microtubule movements in FTD-MAPT neurons that deform the nuclear membrane. This in turn results in a defective nucleocytoplasmic transport. The authors suggest that the presence of tau in the cell body promotes an aberrant microtubule stability, and that this effect leads to increased pushing forces on the nuclear membrane and to the consequent formation of invaginations in the nuclear membrane. Interestingly, acute treatment of FTD-MAPT cortical neurons with the microtubule depolymerization molecule nocodazole reduced the proportion of neurons with nuclear invaginations, restored the round nuclear morphology and restored the nucleocytoplasmic transport to the level of healthy control neurons.

As the rescue of nuclear shape and of nucleocytoplasmic transport *via* microtubule reorganization (by NAT10 tubulin acetyltransferase inhibition) was achieved in another age-related syndrome, the HGPS (Larrieu et al., 2014), it would be of interest to see whether, by exchanging the treatments, the two phenotypes could still be reverted. This would indicate microtubule dynamics as a potential therapeutic target not only for the two cited pathologies, but more generally for any disease with altered nuclear shape and nucleocytoplasmic transport.

In summary, both studies underlie how alteration in the metabolism of microtubule-associated tau protein, a typical hallmark of AD and Tauopathies, is responsible for alteration in nucleocytoplasmic transport. This finding points at microtubule dynamics and stability as a potential pathophysiological mechanism that could be targeted for potential therapeutic interventions in AD, FTD, and other neurodegenerative diseases.

4.3 | FTD and ALS

Other neuropathological disorders markedly linked to nuclear transport alterations are FTD and ALS (Gijselinck, Cruts, & Broeckhoven, 2018). The repeat expansion of the hexanucleotide GGGGCC (G4C2) in a noncoding region of the *C9orf72* gene is the most common cause of sporadic and familial form of these disorders, but the molecular mechanisms of neurodegeneration are quite unknown. While a low number of G4C2 repeats are associated with healthy individuals, an increased repeat number is observed in diseased patients. Different theories attempt to explain the pathogenic mechanisms of the disease: (a) Loss-of-function of the protein (*C9orf72* haploinsufficiency)—the expanded repeats interfere with the physiological transcription or translation of the protein, leading to a decreased expression level of the coded protein; (b) RNA-mediated gain-of-function—expanded repeats form nuclear RNA foci that sequester and deplete essential RNA-binding proteins, leading to neurotoxicity; (c) Protein-mediated gain-of-function—the expanded repeats are translated into a potential toxic dipeptide repeat protein *via* an unconventional translational mechanism (Repeat-Associated Non-ATG initiated (RAN) translation (Zu et al., 2011)) which evades the requirement for a canonical ATG start codon (see for review (Gendron & Petrucelli, 2018)).

In an attempt to investigate and clarify these mechanisms, three different studies discovered a clear association of the disease causing mutation with a compromised nucleocytoplasmic transport. Rothstein's group (Zhang et al., 2015) showed how

C9orf72 repeat expansion affects the function of RanGAP1, similarly to the studies showing that RanGAP1 was altered by mHTT aggregates (Gasset-Rosa et al., 2017; Grima et al., 2017). While the authors previously identified that RanGAP1 interacts with G4C2 RNA, they also showed that RanGAP1 is mislocalized in flies, neurons from *C9orf72* ALS patient-derived induced pluripotent stem cells (iPSC-derived neurons), and in *C9orf72* ALS patient motor cortex and cerebellum tissue. Interestingly, overexpression of RanGAP1 in flies suppressed neurodegeneration, as well as pharmacological enhancing of nuclear import or blockade of nuclear export activity. Similar results were later identified in mouse models that express the dipeptide repeats in their brain (Zhang et al., 2016).

In parallel, Taylor's group (Freibaum et al., 2015) generated transgenic fruit fly lines that expressed 8, 28, or 58 G4C2-repeat-containing transcripts in their neurons. They identified a dose-dependent harmful effect on neurodegeneration. After performing a genetic screen, in which the authors systematically mutated other genes of the fruit fly to identify those genes that ameliorated or worsened the neuronal damage, they identified 18 genetic modifiers encoding components of the nuclear pore complex. Specifically, the loss of two genes, NUP50 and Ref1 (Drosophila orthologs of human NUP50 and ALYREF) strongly enhanced or suppressed, respectively, neurodegeneration in the mutant flies. According to this result, the authors identified morphological abnormalities of the nuclear envelope and defects in RNA export in disease-mimicking neurons.

Another team of researchers led by Aaron Gitler (Jovičić et al., 2015) focused more specifically on the toxicity mediated by the dipeptide repeats. The authors expressed in yeast each of the five dipeptide repeats produced by the unconventional RAN translation of the expanded G4C2. They identified that arginine-containing dipepdite repeats are the most toxic specie. By performing a genetic screen aiming to identify modifiers of the dipeptide repeats-mediated toxicity (similar to the approach used by Taylor's group), the authors identified 11 genes that regulate nucleocytoplasmic transport (including karyopherins and effectors of Ran-mediated nucleocytoplasmic transport). According to these results, the authors identified nucleocytoplasmic transport disturbance in *C9orf72*-ALS patient-derived neurons. A following study substantiated the results of the yeast genes also in drosophila (Boeynaems, Bogaert, Michiels, et al., 2016).

In summary, these three studies all converged on the same genes and pathways and identified that an impairment of nucleocytoplasmic transport is a key factor for toxicity in models mimicking ALS-FTD and suggest therefore that this impairment can be a bona fide contributor to the initiation or to the progression of ALS-FTD disorders in humans.

Another common histopathological hallmark in the brains of ALS/FTD patients is the cytoplasmic mislocalization of the (predominantly nuclear) RNA-binding TDP-43 (transactive response DNA-binding protein, 43 kDa) and the accumulation, in detergent-insoluble protein aggregates, of its aberrantly phosphorylated and ubiquitinated C-terminal fragment (CTF) in neurons and glial cells (Igaz et al., 2008; Neumann et al., 2006). TDP-43 is a 414 amino acid protein that is encoded by the *TARDBP* gene. It contains two RNA-recognition motifs and a glycine-rich C-terminal region that allow it to bind DNA, RNA, and proteins (Buratti et al., 2001, 2005; Wang, Wang, Bose, & Shen, 2004). Remarkably, the C-terminal domain of TDP-43 harbors the majority of the disease-causing mutations responsible for ALS/FTD, which promote the cytoplasmic mislocalization and neurotoxicity of TDP-43 (Gitcho et al., 2008; Guo et al., 2011; Nonaka, Kametani, Arai, Akiyama, & Hasegawa, 2009). Although its physiological functions are still to be fully understood, TDP-43 was shown to regulate gene transcription and mRNA metabolism (from splicing, stability, transport, and translation, to the formation of cytoplasmic and nuclear stress granules) (Li, King, Shorter, & Gitler, 2013; Ratti & Buratti, 2016). Even if an aberrant sequestration and aggregation of RNA-binding proteins could lead to altered RNA and protein homeostasis, the precise molecular mechanism by which TDP-43 cytoplasmic aggregation causes neurodegeneration remains to be elucidated.

In order to address this issue, Ching-Chieh Chou and colleagues (Chou et al., 2018) investigated the composition of detergent-insoluble TDP-43 and TDP-43-CTF aggregates by proximity-dependent biotin identification (BioID). The authors identified that the cytoplasmic aggregates are particularly enriched from components of nucleocytoplasmic transport pathways. Among the 254 TDP-43-associated and the 389 TDP-43-CTF-associated proteins identified, a gene ontology cluster analysis revealed an enrichment in the functional categories of mRNA processing and splicing, intracellular protein transport and translation initiation. Interestingly, the authors identified nucleocytoplasmic transport components as one of the major groups of proteins interacting with TDP-CTF aggregates. By co-expression studies, the authors showed how expression of TDP-43-CTF or of TDP-43 expressing ALS-linked missense mutations caused the mislocalization and cytoplasmic aggregation, to varying degrees, of transcription factors and nucleoporins identified in the interactome screening, and that “prion-like” domains expressed by nucleoporins and transcription factors were responsible for the cytoplasmic aggregation with TDP-43-CTF. Aggregated and ALS-linked mutant TDP-43 were also shown to disrupt NPC and nuclear lamina morphology and nucleocytoplasmic transport, as revealed by a reduced nuclear import of the fluorescent reporter tdTomato protein flanked by NES and NLS sequences and by an increased nuclear retention of poly(A) RNAs in primary cortical neurons. The authors confirmed their findings both in human fibroblasts and in induced pluripotent stem cell-derived neurons. Additionally, *in vivo* studies in Drosophila disease models for ALS showed how loss-of-function mutations in several nucleoporins suppressed retinal degeneration and larval motor dysfunction, two main phenotypes induced by the expression of mutant TDP-43 in Drosophila motor neurons. These results indicate that at least some aspects of TDP-43 toxicity depend on its deleterious effects on nucleocytoplasmic transport machinery also *in vivo*. Nuclear pore pathology was confirmed also in human ALS brain specimens, as evidenced by nucleoporins-positive cytoplasmic inclusions and colocalization with

TDP-43-positive inclusions in motor cortex, hippocampus, and frontal cortex. Finally, the authors show how the suppression of TDP-43 toxicity, either by pharmacological intervention (via inhibition of CRM1-dependent nuclear export) or by molecular intervention (via overexpression poly(A)-binding protein nuclear 1, PABPN1, a potent suppressor of TDP-43 toxicity and aggregation), rescues the defective nucleocytoplasmic transport function.

4.4 | Both disease-associated and non-disease-associated aggregates alter nucleocytoplasmic transport

In line with the finding that protein aggregates can alter nucleocytoplasmic transport and contribute to brain dysfunction, Woerner and colleagues (Woerner et al., 2016) reported that β-sheet-rich aggregates also trigger a similar neurotoxic effect. Using artificial aggregation-prone proteins, as well as authentic disease proteins (fragment of mHTT and TDP-43), the authors show that the aggregates caused the segregation of proteins containing disordered and low-complexity sequences such as nuclear import and export factors (such as THOC2, discussed below). In turn, this aggregation in the cytosol interfered with nucleocytoplasmic transport. Indeed, nuclear transport of proteins was altered (as evidenced by the altered transport of GFP reporter proteins carrying nuclear export (NES) and nuclear localization (NLS) sequence). Additionally, defects on mRNAs transport, and consequently a substantial nuclear accumulation and concomitant cytosolic reduction of mRNAs, were triggered by the presence of the cytosolic aggregates. How do protein aggregates achieve these effects? The authors show how the expression of the protein aggregates in the cytoplasm altered the distribution of NPC proteins, and partially dislocated NPC proteins to the cytoplasmic aggregates. Interactome analysis of the protein aggregates identified how the aggregates sequester several proteins involved in nuclear transport, including importins and components of the THO (suppressor of the transcriptional defects of hpr1Δ by overexpression) complex involved in mRNA export (Olzscha et al., 2011; Woerner et al., 2016). Therefore, as in the case highlighted by Grima and Gasset-Rosa, protein aggregates are shown to alter nucleocytoplasmic transport of proteins and mRNA potentially by binding to proteins associated to the NPC, and by virtue of this, induce the destabilization of the proper nuclear import-export of both proteins and mRNA. As protein aggregates are hallmarks not only of pathological, neurodegenerative brains (Ross & Poirier, 2004), but are also present and produced during normal, physiological brain aging (David, 2012; Grimm, Hoehn, Davies, & Grune, 2011; Kumar, Taha, Sharma, Kale, & Baquer, 2008), the general mechanism described by Woerner and colleagues can explain how an alteration of the proper nuclear-cytoplasmic flow can be triggered both in diseased and in non-diseased state. Moreover, while this mechanism can underlie some aspects of the neuronal dysfunction during healthy aging, it can also be at the base of the worsened, impaired neuronal functioning during pathological aging when neurons are challenged by the additional accumulation of aggregation-prone proteins.

5 | NUCLEAR VERSUS CYTOSOLIC FUNCTION: THE CASE OF MGRN1 IN THE AGING BRAIN

In line with the importance of a correct nuclear import-export for proper neuronal aging is our recent study on the protein Mahogunin (Benvegnù, Mateo, Palomer, Jurado-Arjona, & Dotti, 2017). *MGRN1* gene codes for the protein Mahogunin, a cytosolic CH3hCH4 RING type E3 ubiquitin ligase (Phan, Lin, LeDuc, Chung, & Leibel, 2002). Mutations of this gene in mice are responsible for the mahoganoid phenotype, that is, a complex phenotype that includes a darkening effect on color coat (Phan et al., 2002), an abnormal patterning of the left-right axis (Cota et al., 2006), mitochondrial dysfunction (Sun, Johnson, & Gunn, 2007), and an obesity-rectifying effect on agouti mutant mice (Miller et al., 1997; Overton & Leibel, 2011). Interestingly, mice lacking MGRN1 also show a progressive neurodegenerative phenotype, first apparent in the hippocampus CA3 region at 2 months of age and later extending to multiple regions of the brain (He et al., 2003), indicating that physiological levels of MGRN1 are neuroprotective during adulthood and aging. Moreover, MGRN1 was later described to protect against oxidative and endoplasmic reticulum stress (Chhangani & Mishra, 2013), against cytosolic prions (Chakrabarti & Hegde, 2009) and polyglutamine neurotoxic aggregates (Chhangani et al., 2014), confirming that MGRN1 levels are crucial to provide general neuronal protection. We later described how MGRN1 levels can also be crucial for controlling the secretion of β-amyloid peptides, the main component of amyloid deposits identified in the brains of AD patients (Benvegnù, Wahle, & Dotti, 2017). Starting from these observations, we analyzed a potential role for MGRN1 during aging of the animal and identified that MGRN1 levels chronically decrease with aging in the hippocampus of mice. Moreover, during aging MGRN1 relocates to neuronal nuclei. The cytosol-to-nucleus shift of MGRN1 is due to a decline in proteasomal activity, a typical event occurring with brain aging (Ding et al., 2002; Keller et al., 2002; Rousseau & Bertolotti, 2018; Schmidt & Finley, 2014). The decline in proteasomal activity induces the accumulation of protein aggregates (Saez & Vilchez, 2014). Therefore, considering the altered nucleocytoplasmic transport triggered by the presence of protein aggregates (as described above) typical of the aging brain, we cannot exclude that nuclear accumulation of MGRN1 can be additionally influenced by the presence of such aggregates. Once in the nucleus, MGRN1 associates with active chromatin and with the transcription factor ATF3 (Activating Transcription Factor 3), a main core point of the cellular adaptive-response network to stress that is expressed in response to different types of stressors and provides an adaptive transcriptional response by binding to the promoter of target genes and regulating their transcription (Hai, Wolford, & Chang, 2010). Nuclear MGRN1, by interacting with ATF3 and possibly preventing its degradation, potentiates the transcriptional cellular response to proteasomal stress, and strengthens neuronal functionality. Proteasomal dysfunction, and in general the perturbation of protein homeostasis and of

the clearance mechanisms for damaged and misfolded proteins (Vilchez, Saez, & Dillin, 2014) can therefore have different consequences: (a) on one side, it induces the accumulation of neurotoxic aggregates in neurons that interfere with nucleocytoplasmic transport (as described) consequently leading to altered subcellular accumulation of proteins; this altered subcellular localization can, in turn, lead to neuronal loss of homeostasis; (b) on the other side, the novel, altered localization of such proteins (as is the case of nuclear MGRN1 after proteasomal impairment) can be an adaptive, compensatory response of the cell to a proteotoxic challenge.

MGRN1 likely plays a dual role as a component of the neuron homeostasis control system: one in the cytosol, as in young animals, to directly challenge cytotoxic aggregates and toxic stress; and one in the nucleus, in old animals, where in virtue of its "novel" localization in active chromatin regions it can participate in the orchestration and potentiation of the cellular response to proteotoxicity at transcriptional level. By increasing the levels of MGRN1 *in vitro* and *in vivo*, we could ameliorate signs of neuronal stress and aging, including cognitive decline in aged mice. MGRN1 cytosol-to-nuclear trafficking appears therefore not to be a toxic phenomenon, but rather an active mechanism of the cell in order to build up a proper response against stressful, toxic stimuli. A similar mechanisms is described for Nrf2, a nuclear transcription factor controlling the coordinated expression of a set of protective genes encoding antioxidant proteins and detoxifying enzymes (Ma, 2013). Nrf2 is excluded from the nucleus by the major exportin CRM1. However, oxidants can modify and inactivate CRM1 function. The inactivation of CRM1-mediated nuclear export consequently facilitates nuclear entry and accumulation of Nrf2 and transcriptional activation of Nrf2-controlled genes, resulting eventually in an increase in the cellular antioxidant capability (Wang et al., 2009).

These findings rely on different mechanisms from the ones occurring, for example, with mHTT (Gasset-Rosa et al., 2017; Grima et al., 2017), where nuclear accumulation of cytosolic proteins and mRNAs seems to be an uncontrolled event due to aging and, in an exacerbated manner, to Huntington's disease state. Such nuclear accumulation of cytosolic proteins and of mRNAs seems indeed to be nonfunctional, differently from the nuclear accumulation of cytosolic MGRN1 observed with aging, where the nuclear accumulation of a cytosolically localized protein seems to be, on the contrary, an adaptive and neuroprotective response. One possible scenario explaining this distinction can be a differential mechanism for the nuclear accumulation of proteins, being this mechanism uncontrolled in the case of protein aggregates and the consequent alteration in nucleocytoplasmic transport, while regulated in the case of MGRN1 nuclear entry. On the other hand, an uncontrolled nucleocytoplasmic transport can lead to the nuclear accumulation not only of potentially toxic and aggregated proteins, but also of functional proteins that can exert their functions, as is the case of MGRN1 with ATF3, in a novel environment. In this perspective, additional studies need to clarify the nature and the potential "nuclear functionality" of proteins, normally localized in the cytosol in basal conditions, that accumulate in the nucleus as a consequence of the alteration of the (active and passive) nuclear transport observed in aging and in diseases.

6 | GLUCOCORTICOID RECEPTORS NUCLEAR ENTRY IS ALTERED IN BRAIN AGING

Additional proteins that exhibit altered nucleocytoplasmic transport during brain aging are the glucocorticoid receptors (GRs). The glucocorticoid receptors are intracellular receptors expressed virtually in all cells in the body. GRs bind to glucocorticoids which are secreted by ACTH-stimulated adrenal glands when the brain perceives a stressor (known as the hypothalamic-pituitary-adrenal (HPA) axis stress response (Smith & Vale, 2006)). In the absence of the hormone, the GR basally resides in the cytosol of the cell thanks to the help of several chaperone proteins (Pratt, Morishima, Murphy, & Harrell, 2006). Once cortisol diffuses through the plasma membrane and binds to GR, this results in the release of the chaperone proteins, consequently allowing the entry of the GR inside the nucleus. In the nucleus, GRs bind to specific DNA responsive elements and either activate or repress gene transcription (Weikum, Knuesel, Ortlund, & Yamamoto, 2017). In the brain, GRs are expressed ubiquitously and can function as nuclear activators or repressors. A tonic production and a transient increase in glucocorticoids during periods of stress are essential for neuronal plasticity and normal brain function (Aguilera, 2011). As GR main signaling pathway is via nuclear entry and DNA binding, it is not surprising therefore that the interference with these two phenomena can lead to neuronal dysfunction. The activity of HPA axis during aging increases, as a consequence of an inefficient glucocorticoid negative feedback inhibition (Mizoguchi et al., 2009). One major consequence of this decrease in glucocorticoid negative feedback inhibition is an altered GR signaling in neurons (Lee, Hwang, Yun, & Han, 2012), with consequences on neuronal survival and functionality, and in turn in memory (He et al., 2008; Hibberd, Yau, & Seckl, 2000). Mutations in GR that prevent DNA binding are associated with impaired spatial memory (Oitzl, Reichardt, Joëls, & Kloet, 2001). Importantly, a decreased DNA-binding activity and a diminished nuclear entry of GR are described in neuronal aging (Murphy, Spencer, Sipe, & Herman, 2002). Moreover, GR levels were described to decrease only in the nucleus, but not in the cytosol, of hippocampal neurons of old rats with spatial memory impairment, with a consequently decreased GR DNA-binding activity (Lee et al., 2012). These observations suggest that the reduced nuclear levels of GR in old neurons can be the consequence of an altered nucleocytoplasmic transport of GR. Upon binding to ligands, GR nuclear import through the nuclear pore is normally controlled by the interaction of nuclear pore complex proteins such as importins (Freedman & Yamamoto, 2004; Hakim, Barnes, Adcock, & Usmani, 2013; Tao, Lan, Lukacs, Haché, & Kaplan, 2006) and nucleoporins (Echeverría et al., 2009) with GR-associated chaperone proteins (Davies, Ning, & Sánchez, 2002; Echeverría et al., 2009; Galigniana, Radanyi, Renoir, Housley, & Pratt, 2001; Wochnik et al., 2005). While an extensive

number of studies investigated the details of GR nuclear import in cells, an exhaustive description of the mechanisms leading to the decreased nuclear entry of GR as observed in neuronal aging is still missing. Nevertheless, this effect can be explained by a decrease in the expression levels, with aging, of chaperone proteins that are responsible for GR nuclear translocation (Murphy et al., 2002). In fact, chaperones proteins HSP90 and the constitutively active HSC70 are essential components of the chaperone complex (Rajapandi, Greene, & Eisenberg, 2000), which allow for high-affinity glucocorticoid binding to GR (HSP70) and for the proper folding of the complex (HSC70). Aged rats showed marked decreases in hippocampal HSP90 and HSC70 levels in both nuclear and cytoplasmic extracts (Murphy et al., 2002). This decrease in chaperone proteins levels can therefore eventually participate in the altered glucocorticoid signaling described in hippocampal aging. In summary, the inappropriate HPA axis and GR signaling that occurs with brain aging can be, at least in part, due to age-dependent inefficiency in the nuclear translocation of GR. This GR-dependent altered signaling with aging is not only relevant for age-dependent neuronal alterations, but can improperly affect a health aging process and increase adverse effects in stress-related brain disorders (Aguilera, 2011).

7 | CONCLUSIONS

Brain aging is characterized by progressive changes in several neuronal molecular and cellular processes, among which a disturbance of nucleocytoplasmic transport and the presence of protein aggregates. These two features are observed during both healthy physiological brain aging and in neurodegenerative diseases, such as Huntington's disorder, AD, C9orf72-linked, and TDP-43-linked FTD-ALS. Mechanistically, protein aggregates trigger the dysfunction of the nucleocytoplasmic transport, which in turn results in the additional accumulation of subcellularly mislocalized proteins, and in a consequent cellular dysfunction. As both non-disease-associated and disease-associated proteins can alter the nuclear pore complex components and follow the same fate, these studies indicate therefore that alterations of nucleocytoplasmic transport and of its components are a common, shared phenomenon between physiological and pathological brain aging (Figure 1).

Moreover, the parallels evidenced in these reports between neurons that are in a diseased, neurodegenerative scenario (i.e., HD, Mahoganoid mice, FTD-ALS, …), and wild-type aging neurons, they all suggest that: (a) the maintenance of a correct nuclear import–export trafficking (and a potential therapeutic intervention for rescuing a dysfunctional nuclear transport) is pivotal for maintaining a physiological neuronal homeostasis and functionality; and (b) "pathological aging" can be a form of "physiological aging" accelerated and exacerbated by some genetic factors and/or disease mechanisms that perturb nucleocytoplasmic transport and maintain and extend neuronal dysfunction till the onset of a disease state.

Is there a common mechanism that links physiological aging to neurodegeneration? The question is still to be resolved; nevertheless, oxidative stress could be one of these mechanisms. In regards to physiological aging processes, an increase in oxidative stress biomarkers was shown to correlate with high levels of inflammatory cytokines, and both indicators were associated with low cognitive performance in institutionalized elderly persons (Baierle et al., 2015). Coherently, cognitive impairment was shown to be slower in old patients with high glutathione peroxidase antioxidant activity (Revel et al., 2015). Reactive oxygen and nitrogen species (RONS) accumulate during physiological aging, inducing oxidative damage to macromolecules such as lipids, DNA, and proteins (Beckman & Ames, 1998; Liguori et al., 2018), and NPC is no exception (D'Angelo et al., 2009). Oxidative damage to proteins is also responsible for protein aggregation during physiological aging (Squier, 2001). Therefore, oxidative stress can impair NPC function both directly, *via* oxidant damage to NPC components, and indirectly, *via* protein aggregates-mediated inhibitory effect on NPC activity (Figure 1, central part). As in aging the oxidative stress is a chronic condition, there is an enhanced amyloid-like aggregation affecting both non-disease-associated and disease-associated proteins (Chen & Liu, 2017). Additionally, the presence of non-disease-associated protein aggregates, or the presence of pathogenic mutations, together with the persistent oxidative stress accompanied by aging, can accelerate a transition to a neuropathological scenario, where protein aggregates (both disease-associated and non-disease-associated) and RONS furthermore deteriorate and aggravate NPC functions (Figure 1, right part). Mitochondria are one of the main sources of ROS during aging (Cui et al., 2012; Stefanatos & Sanz, 2018). Therefore, any age-related mechanism interfering with mitochondria metabolism can be potentially connected also to oxidative stress production. Can MGRN1 be a link between brain aging and ROS production? Mice lacking MGRN1 present mitochondrial dysfunction months before the onset of neuronal degeneration (Sun et al., 2007). Additionally, MGRN1 was shown to regulate mitochondria dynamics (i.e., fusion), and that loss of MGRN1 levels induces an increase in ROS levels in HeLa cells (Mukherjee & Chakrabarti, 2016). We showed how loss of MGRN1 increases ROS levels also in primary hippocampal neurons (Benvegnù, Mateo, et al., 2017). As overall MGRN1 brain levels decrease with aging (Benvegnù, Mateo, et al., 2017), this phenomenon can in turn affect mitochondria homeostasis and consequently rise ROS production, and be one of the age-related mechanisms responsible for increased oxidative stress that occurs during neuronal aging.

An additional challenging question is whether and how nucleocytoplasmic transport should be therapeutically targeted, given the high complexity of this process. Different strategies were shown to be beneficial to rescue neuronal death, nucleocytoplasmic transport defects, and nuclear morphology:

- *Nuclear import*:

Defects in nuclear import play a causative role in the pathogenesis of ALS/FTD (Boeynaems, Bogaert, Van Damme, & Van Den Bosch, 2016; Kim and Taylor, 2017) and polyQ diseases similar to HD such as spinocerebellar ataxia type 3 (SCA3) (Sowa et al.,

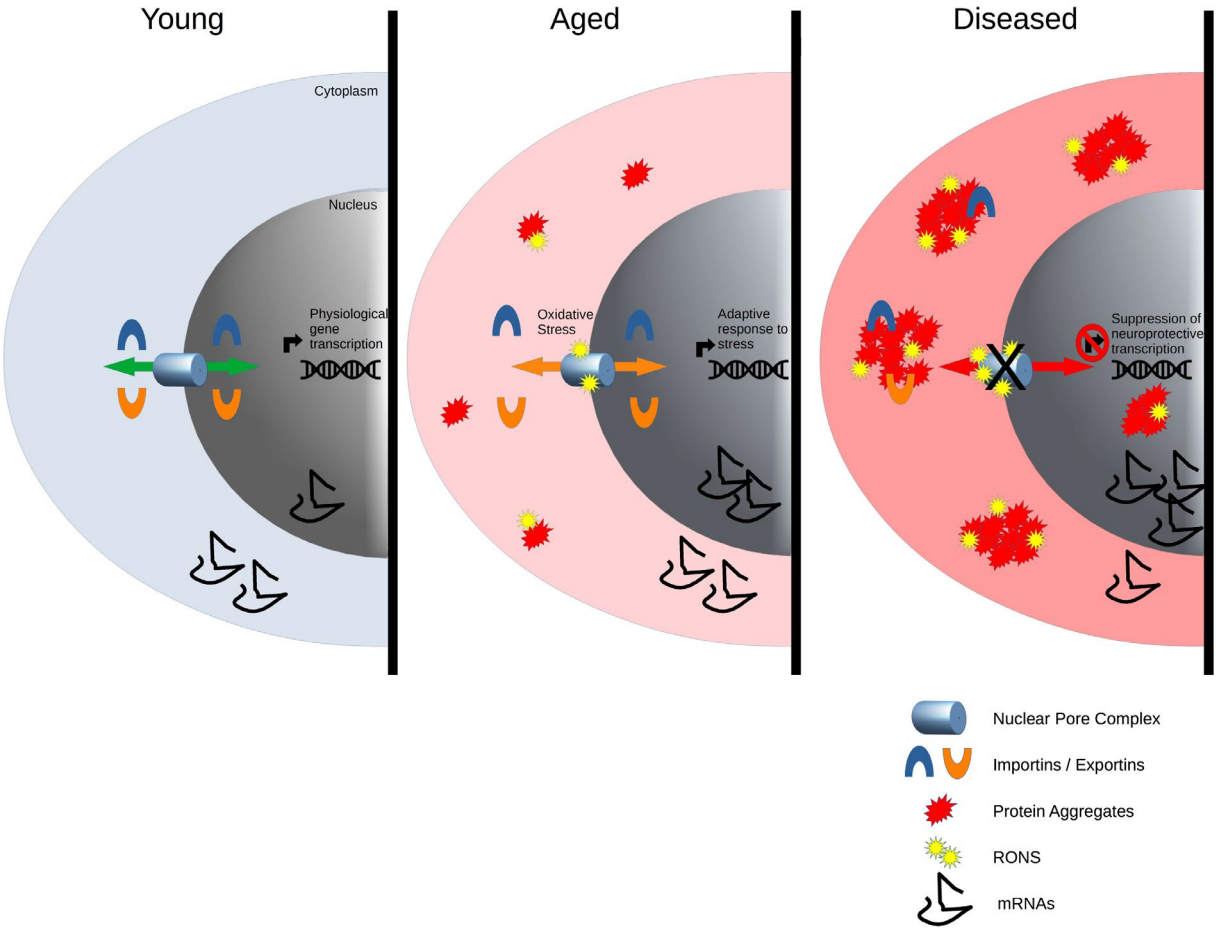

FIGURE 1 Alteration of nucleocytoplasmic transport in brain aging and disease. In young neurons (left panel), oxidative stress and protein aggregates are minimal. The nucleocytoplasmic transport is therefore unaffected and properly functioning. This allows the physiological compartmentalization of both proteins and mRNAs. In aged neurons (central panel), the increase in reactive oxygen and nitrogen species (RONS) induces oxidative damage directly to the NPC. Additionally, the age-dependent perturbation of proteostasis, together with the increase in oxidative stress, can favor the insurgence of both disease-related and non-disease-related protein aggregates. These aggregates in turn affect the functionality of NPC, altering the proper nucleocytoplasmic compartmentalization. Neurons undergo a transcriptional response to age-related stress (such as proteotoxic and oxidative stress), to counteract neuronal dysfunction and maintain homeostasis. However, a prolonged exposure to additional adding-up stress can overwhelm the cellular capacity to elicit a proper transcriptional response—leading eventually to cellular damage and neurodegeneration. Indeed, in neurodegenerative brains (right panel), the presence of protein aggregates sequesters and alters the localization of importins and exportins, as well as of other nuclear transport factors and of transcription factors. Additionally, the protein aggregates can directly interact with the NPC, leading eventually to the disruption of the NPC functions and to the final imbalance of nucleocytoplasmic transport of both mRNAs and proteins. The aberrant nucleocytoplasmic compartmentalization and sequestration in protein aggregates of transcription factors and regulatory proteins affect nuclear transcriptional response, leading in turn to the suppression of neuroprotective transcription and, eventually, to loss of homeostasis and cell death

2018). While in some cases overexpression of importins was shown to be beneficial and neuroprotective (Guo et al., 2018; Jovičić et al., 2015), in other cases, depletion of importins rescued neurodegeneration (Sowa et al., 2018). The pathogenic mechanisms at the bases of the different diseases are still to be fully elucidated, and so the potential pharmacological intervention. Nevertheless, importins can become an important therapeutic target not only for cancer therapy (Hill, Cautain, de Pedro, & Link, 2013), but also for neurodegenerative disorders.

- *Nuclear export*:

Partial inhibition of the exportin CRM1 was found beneficial in fly and neuronal models of C9orf72 (Zhang et al., 2015), in TDP-43-linked FTD (Chou et al., 2018), and in Huntington's disease (Grima et al., 2017) by rescuing nuclear morphology defects and neuronal death (as previously discussed in the text). The inhibition of nuclear export activity can confer neuroprotection by compensating the defects in nuclear import under different pathological conditions.

- *O-GlcNAc transferase inhibition*:

Huntington's disease-mediated neuronal death, Ran localization, and nucleocytoplasmic transport defects were also rescued with an inhibitor of O-GlcNAc transferase (O-GlcNAcase, OGA). O-GlcNAcylation is a posttranslational modification in which β-N-acetylglucosamine (GlcNAc) is added to intracellular proteins (Zachara and Hart, 2006), and it is essential for NPC integrity and function as well as for the maintenance of the pore selectivity filter (Zhu et al., 2016). Moreover, O-GlcNAc is significantly decreased in mHTT-expressing cortical cells, indicating that nucleoporins mislocalization could be due to decreased levels and/or misregulation of O-GlcNAc. Inhibition of OGA resulted in elevated levels of O-GlcNAc with consequent reduction of neuronal death and rescue of Ran localization and nucleocytoplasmic transport in mHTT neurons.

- *Microtubule stability intervention*:

Nuclear shape and nucleocytoplasmic transport were rescued by intervention on microtubule dynamics, with nocodazole in Tau-linked FTD (Paonessa et al., 2019) and with a small NAT10 inhibitor molecule in HGPS (Larrieu, Britton, Demir, Rodriguez, & Jackson, 2014, Larrieu et al., 2018). The alteration in microtubule network and stability in the diseases deforms the nuclear membrane and the nuclear shape. The pharmacological intervention aimed at a microtubule reorganization is suggested to release mechanical forces acting on the nucleus, thereby contributing to a normalization of the nuclear shape and nuclear functions.

Given these several options for intervention, it would be of extreme interest to understand the efficacy of the different treatments and interventions. For example, could these different pharmacological treatments be added up for an increased rescue activity in case of disease? How disease-specific must be a multidrug approach? Moreover, as these interventions have been made in different laboratory species, cell lines and cultures, is there a limit for our intervention depending on the laboratory disease models used? Although there is still so much to be learned to answer these questions, nevertheless this is a quickly moving field, and these issues will be surely addressed in the near future.

Additional questions remain. For example, the relative roles and interplays between the different neurotoxic aggregates and species causing damage to the nucleocytoplasmic transport are not yet resolved. Do their actions add up? Additionally, how the impaired nucleocytoplasmic transport affects other neuronal functions during aging is still unresolved. Why, in case of NPC dysfunction, are some proteins more affected in their subcellular compartmentalization, while other are more spared? Moreover, are all mislocalized proteins toxic to the neuron? Or, as the case of MGRN1, can the newly localized protein acquire or perform novel functions in a novel environment? If so, are these novel functions protective for the neurons? In addition, is there a potential therapeutic intervention on this system that can be undertaken to strengthen neuronal functionality during aging and, consequently, to potentially prevent neurodegenerative scenarios? The answers to these issues will shed additional light on mechanisms underlying all these processes and will indeed advance our understanding on different neurodegenerative disorders.

CONFLICT OF INTEREST

Stefano Benvegnù declares that he has no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, S.B.; *Writing—original draft*, S.B.; *Writing—Review & Editing*, S.B.

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